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**LARGE SCALE BREATHE MONITORING FOR ASTHMA PHENOTYPING**

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**Introduction**

Asthma is one of the most prevalent chronic disorder worldwide, affecting 235 million people. This represents a serious public health issue associated with high health costs, mainly due to the diagnosis and treatment. A European study has estimated the cost of asthma to 19.3 billion euros/year. Asthma is characterized by an inflammation of the airways, involving several underlying mechanisms. Induced sputum is the gold standard method to assess bronchial inflammation. However, this approach is invasive and not widely available. In addition, current therapies remain ineffective in a large proportion of patients. For these reasons, the characterization of different inflammation phenotypes (i.e.
eosinophilic, neutrophilic, paucigranulocytic, mixed-granulocytic asthma) is of great importance to provide personalized treatment.

Methods

Volatile organic compounds from breath of 245 asthmatic patients covering a range of four different asthma phenotypes were analyzed. The breath samples were collected into 5 L Tedlar® bags. Thermal desorption coupled with comprehensive two-dimensional gas chromatography (GC×GC) – high resolution time-of-flight mass spectrometry was applied for the analysis. In this context of complex mixtures analysis, GC×GC offers a powerful solution to obtain a complete overview of the samples’ composition. To handle the complexity of generated data, quality control (QC) protocol was established to insure the proper use of the analytical instrument. Internal standards were injected on a regular basis, every 30 samples, to monitor analytical variations, i.e. 1st and 2nd retention times and VOCs intensities.

Preliminary Data

The data were pre-process using large scale alignment tool. Subsequently, the data were split between training and test (60-40%). Random forest algorithm was used to investigate the ability of exhaled breath VOCs to distinguish between the inflammatory profiles. The random forest algorithm was built on 7 significant features highlighted in a first discovery study performed on an independent cohort of 276 patients. ROC curves were constructed to evaluate the classification performance in pair-classification scenario. The AUROC classifications reached 0.71, 0.68 and 0.70 with 70%, 60% and 64% of accuracy. The prediction accuracy using VOC measurement was similar to the accuracy of blood eosinophil levels and exhaled nitric oxide (FeNO) levels that are commonly used in clinical practice. In addition, the combination of
blood eosinophil counts, FeNO and VOCs measurements gave the best prediction accuracy of 76%. VOCs also enabled the identification of sputum neutrophilia in the whole population of asthmatics with similar statistical power than isolated markers (FeNO, blood eosinophils) used to predict sputum eosinophilia. Possible confounding factors such as smoking, inhaled corticosteroid usage, age and BMI were evaluated, and no interference was highlighted. This first large-scale confirmatory study shows that breath VOCs analysis is an efficient approach for asthma phenotyping and is going to lead to further development in clinical diagnosis.

**Novel Aspect**

This work is a step forward for designing exhaled air inflammometers as clinician use glucometers in diabetes, to adapt treatments.

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